



## Research article

# Improving water productivity in the hydroponics with a plasma-nanobubble hybrid technology

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## ABSTRACT

Among new technologies aimed at improving water productivity, nanotechnology have been identified as effective means of enhancing the properties of agricultural water. Building on the synergy of plasma and nanobubbles, this study explored the combination of plasma electric discharge and nanocavitation as a novel approach for soilless cultivation. The study aimed to investigate the impact of this hybrid technology on hydroponics nutrient solution. Additionally, the study further aimed to assess the effect of both technologies individually, as well as various application periods, including 3, 9, and 15 min for hybrid technology use. The study employed the nutrient flow technique to hydroponically cultivate lettuce. The findings showed that the application of each technology individually did not significantly increase yield. However, the hybrid technology treatment for 9 min resulted in a significant yield increase of almost 60 %. This improvement can be attributed to the stability, solubility, and absorption of products resulting from plasma treatment, as well as the antimicrobial and anti-algae effects of both technologies. Additionally, an increase in flavonoid and potassium content and a decrease in iron were observed in plants grown under optimal treatment. Overall, this study demonstrated the potential for synergy between plasma and nanobubble techniques in hydroponic culture.

## 1. Introduction

In order to meet water needs in a region or country, the lack of sufficient available freshwater resources is referred to as water shortage [1]. For countries with water scarcity problems, the increase in agricultural water efficiency has a crucial role to play.

In recent years, nanotechnology has been used as a new technology in the food and agricultural industries, and one of the applications of nanotechnology is the creation of nanobubbles in the liquid phase. Nanobubbles are small bubbles of gas with dimensions less than 200 nm and are produced through various technologies such as cavitation, electrolysis, membrane method, etc., among which, the hydrodynamic cavitation method has been introduced as one of the most efficient technologies for the production of nanobubbles [2]. The effect of nanobubbles in improving plant growth has also been reported in some studies [3,4]. Also, one of the mechanisms of the effect of nanobubbles is to increase the amount of water absorption by seeds through increasing the permeability of its coating, which was observed in radish [5]. This technology can maintain high levels of dissolved oxygen in the aqueous phase compared to normal aeration and lead to an increase in plant yield in hydroponics and intensive agriculture [6]. The unique properties of nanobubbles can have positive effects on the plant yield. Due to high surface area and long lifetime, nanobubbles can help increase

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dissolved gases levels in water, which are beneficial for enhancing agricultural products. Recent researches have shown the use of nanobubbles in improving plant growth [7].

On the other hand, cold plasma is a technique used to create chemical compounds and change properties in agricultural water. Water treatment with cold plasma, whose product is known as plasma activated water (PAW), creates an acidic environment that leads to a change in electrical conductivity and oxidation and regeneration potential, as well as the formation of reactive oxygen species (ROS) and nitrogen (RNS). Therefore, plasma-activated water has different chemical compositions and properties compared to water and can have applications such as inhibiting pathogens or improving the germination and growth of plants [8]. Several studies reported increased plant growth, improved stress tolerance, and antimicrobial effects of plasma treatment and plasma-treated water, and it seems that a basis for designing plasma-based fertilization strategies is being provided [9]. Plasma activated water has been used in the agricultural industry to increase crop production. As an example, the improvement of seed germination and plant growth of wheat [10] and chickpea [11] has recently been observed through PAW. The use of this technology in agricultural production should emphasize its systematic nature, controllability and performance and make it practical [12].

The integration of hydrodynamic cavitation and plasma discharge creates a very reactive environment and without the use of chemicals, it is possible to use this dual method (hybrid) for the industrial treatment of polluted water [13]. As the gas bubbles enter the liquid, there is an opportunity to produce a discharge plasma inside the small bubbles and the liquid at the same time. It is also possible to increase the emission of short-lived chemical species in this way [14]. Also, cavitation, in addition to producing chemically active OH radicals, can help purify water pollutants [15].

Studies have shown that due to the characteristics and advantages of nanobubbles, their combination with plasma reactions has led to good results in water purification, however, despite the results of these studies, practical reports have not yet been widely conducted on the combination of plasma technology and nanobubble technology [16].

In a study, the simultaneous treatment of contaminated water with plasma and hydrodynamic cavitation has been investigated. The proposed method showed great potential in industrial applications for water disinfection and purification from certain organic pollutants [13].

Another study reported that increasing the electrical discharge time in both cases with bubbles and without bubbles led to an increase in nitrate concentration and electrical conductivity, but these values were higher in the sample containing micro/nano-bubbles. This method can be used to form more  $H_2O_2$  and  $NO_3^-$  [14].

A new method was also presented for the removal of cyanobacterial biomass during pretreatment of drinking water, which combines hydrodynamic cavitation with cold plasma discharge. Cavitation creates a compressive stress that causes the microcystis to collapse. The hydrogen peroxide produced by the plasma and inside the bubbles has a negative effect on the photosynthetic activity of cyanobacteria [17].

With the aim of expanding the treatable range of water pollutants, a new wastewater treatment process through the synergy of ultrasound radiation and plasma discharge has also been proposed. Ultrasonic radiation into the liquid causes acoustic cavitation. The results showed that plasma production, with the help of ultrasound, requires a lower output voltage and significantly expands the range of wastewater that can be treated with plasma according to its chemical composition and physical properties [15].

A study was conducted with the aim of analyzing the hydrodynamic characteristics and testing some important physicochemical parameters of a plasma-nanobubble reactor. The purpose of producing nanobubbles was to increase plasma absorption by water. The results showed that the solubility of oxygen and ozone, the production of  $H_2O_2$  and the synergism of these species were strongly affected by the electric voltage, the flow rate of the input gas and the purity of  $O_2$  used in the injection system [16].

Plasma and nanobubble have been combined in recent years, which were mainly intended to treat water and wastewater. This method showed good potential for pollution problems and prevention. It has not yet been assessed whether the combination of these two technologies can be advantageous in agriculture. According to the existing findings that were reviewed, this issue is worth investigating. The purpose of this study is to explore enhancing productivity of hydroponics nutrient solution using plasma-nanobubble treatment.

## 2. Materials and methods

### 2.1. Hydroponic cultivation

In this research, applying dielectric barrier discharge plasma (DBD) to air and transforming it into nanobubbles in hydroponic nutrient solution was investigated. Also, the use of plasma and nanocavitation and hybrid technology including these two methods were investigated. Hydroponic cultivation was done based on the nutrient flow technique (NFT), which is a commercial method. In this method, the nutrient solution flows in the channel and after passing through the roots of the plants, it is returned to the tank and used again.

### 2.2. Plant samples

In order to prepare plant samples, romaine lettuce (*Paris Island*) seeds were grown in a seedling tray and inside a mixture of perlite and cocopeat. During the cultivation in the hydroponic system, the seedlings were kept in water for a short time to separate the roots from cocopeat and perlite. After washing them, the seedlings were dipped in a 1:000 captan fungicide solution and the roots were passed through the holes at the bottom of small plastic mesh pots and hydroton was used to keep the plant in the pot.

### 2.3. Hydroponic nutrient solution

Nutrient solution was prepared according to Hoagland and Arnon nutrient solution formulation with minor changes [18]. Table 1 shows the method of preparing stock solutions of macroelements and microelements to prepare full Hoagland solution.

According to the amount of Hoagland solution needed in each stage of the experiments, the degree of dilution and the amount of stocks were determined in such a way that to prepare 1 L of Hoagland solution, 4 cc of each of the macro element stocks, 2 cc of iron stock and 1 cc of micro element stock is enough.

### 2.4. Hydroponic cultivation

Fig. 1 shows a schematic view of a hydroponics unit that was used based on nutrient flow technique.

A special gutter was used as a culture bed and channel for the flow of nutrient solution. The surface of this gutter has shallow grooves that can help to spread water better on the inner floor of the channel. The length of each cultivation bed was considered 1 m. The width and height of the cut section were approximately 8 cm and 6 cm, respectively. Both sides of the gutter were covered with caps. In order to place the pots containing plants, five holes were considered on the upper surface of each channel. The center distance of the holes on each row was about 15 cm and the distance between the rows was about 12 cm. Hydroponic net pots containing plants and hydroton (Expanded clay pellets as a neutral support substrate) were placed inside these holes. These basket-shaped plastic pots provide suitable conditions for root passage. At the inlet of the gutter, a flow control valve was attached to the cap. The nutrient solution was transferred from the hydroponics reservoir to the inlet valve through a polyethylene hose. The other end of the hose was connected to a water pump placed inside the reservoir. The water from the gutter outlet flowed into the tank through a knee pipe, which also helped aerate it.

In order to install gutters and lighting system, a structure was made using perforated corners. To make the slope of the ducts by 1–2%, bolts and nuts were used under the entrance part of the gutter. Artificial light was provided through LED. The luminous efficiency of each tube was 95 Lm/W and its color temperature was 6500 K. The light duration, which was considered to be approximately 16 h per day, was controlled by a mechanical timer.

### 2.5. Applying plasma to the injected gas of the nanocavitation reactor

Fig. 2 shows the schematic of the system that was used to apply plasma to the injected gas of the nanocavitation reactor. In order to apply the dielectric barrier discharge plasma to the air, two steel electrodes and one brosilicate or pyrex dielectric were used, all three having a cylindrical shape. The dielectric thickness was 1.5 mm. Air was supplied by an air pump and after treatment in the plasma reactor and passing through a flowmeter, it was injected into the nanobubble reactor through the gas inlet. The gas flow was around 100 ml/min. The input voltage to the plasma reactor was 220 V with a frequency of 50 Hz. The output voltage applied to the high voltage electrode was a maximum of 3.6 kV with an approximate frequency of 7.5 Hz.

### 2.6. Experimental treatments

According to Fig. 3, six middle channels were used in the experiment to investigate the effect of nanobubble and plasma technologies. The six investigated treatments include 1) control (nutrient solution without treatment), 2) applying DBD plasma to air and injecting it into the solution for 9 min, 3) applying nanocavitation to the solution for 9 min, 4) applying plasma and nanocavitation as a hybrid technology to solution for 9 min, 5) apply hybrid technology for 3 min and 6) apply hybrid technology for 15 min. Therefore, in addition to studying the effect of hybrid technology and comparing it with the control, the effect of both technologies alone as well as the effect of different times of application of hybrid technology were also tested. It is worth mentioning that in order to create ionized air around the liquid when the nanobubbles start to form, the plasma application was started 1 min before the start of the nanocavitation reactor. That is, it can be said that the durations of plasma application in hybrid treatments were 4, 10, 16 min. In order to

**Table 1**

The formulation of the hydroponic nutrient solution.

Component	Stock Solution	ml Stock Solution/1 L	Amount of Substance
			(mMol/L)
Macronutrients			
2 M Ca (NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O	472 g/L	2.5	5
2 M KNO <sub>3</sub>	202 g/L	2.5	5
1 M KH <sub>2</sub> PO <sub>4</sub>	136 g/L	1	1
2 M MgSO <sub>4</sub> ·7H <sub>2</sub> O	493 g/L	1	2
Iron Chelate	15 g/L	1	0.03
			(μMol/L)
Micronutrients			
H <sub>3</sub> BO <sub>3</sub>	2.86 g/L	1	46
MnSO <sub>4</sub> ·H <sub>2</sub> O	1.5 g/L	1	8.88
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.22 g/L	1	0.77
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.025 g/L	1	0.1
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.1 g/L	1	0.4

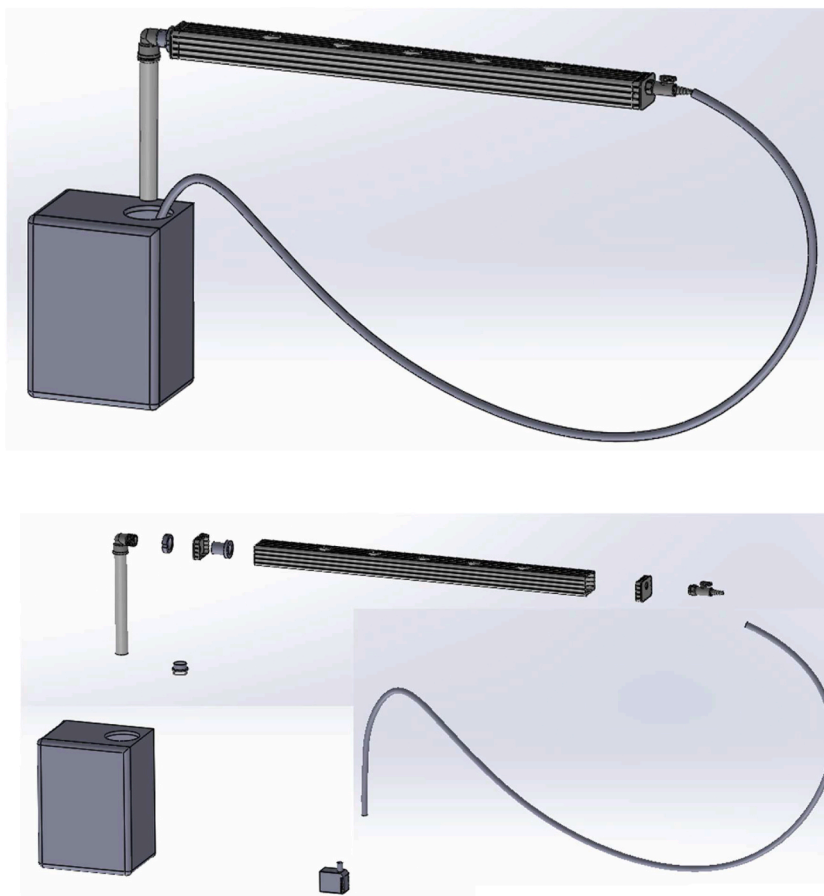


Fig. 1. NFT culture unit components, top: assembled and bottom: exploded design.

correct the comparison of the plasma application treatment alone (treatment #2) with the hybrid method, the duration of this treatment was also considered 10 min.

For each treatment, five replicates or five plants were considered. The volume of the nutrient solution of each unit was 8 L at the time of cultivation, and every week the reservoir solution was drained and 6 L of new solution was added after the treatment. The cultivation period was also 35 days.

## 2.7. Evaluation of results

After the experiment, the number of leaves, stem fresh weight, root fresh weight, total fresh weight, stem dry weight, root dry weight, total dry weight and root to stem dry weight ratio were measured. To measure the dry weight, the samples were dried in an oven at a temperature of 70 °C. In the experiment with the NFT method, the color indices L, a and b were measured. The device used for this experiment was a colorimeter (TES-135 A; TES Electric Electronic Corp. Taiwan). Color measurement was also done in three distinct points of lettuce. The system or color space considered was  $L^*a^*b^*$ . The reason for choosing this scale was that, unlike RGB and CMYK color spaces, Lab is designed to approximate the human eye [19] and since it is intended to be consumer friendly, this scale is more suitable for evaluating the color and appearance of fruit. Also, in this research, after determining the optimal treatment, a comparison was made between the nutritional quality of lettuce grown with this treatment and the conventional or control method. For this purpose, samples were taken from the dry leaves of lettuce and flavonoid as a valuable compound, potassium as an important mineral element and iron were measured in lettuce. To measure the flavonoid, the sample was first extracted. Then, two percent aluminum chloride reagent was added to 5 cc of the 5 cc extract and the absorbance was read at 408 nm. Quercetin was used as a standard and a calibration curve was drawn. Potassium was also measured based on the ISIRI 11114-3 standard and the results were reported as 100 cc/meq. The reference method for measuring iron was ISIRI19719.

The results were analyzed by ANOVA method and comparison of means was done by Duncan's test at a significance level of 0.05. SPSS version 21 software was used for statistical analysis.

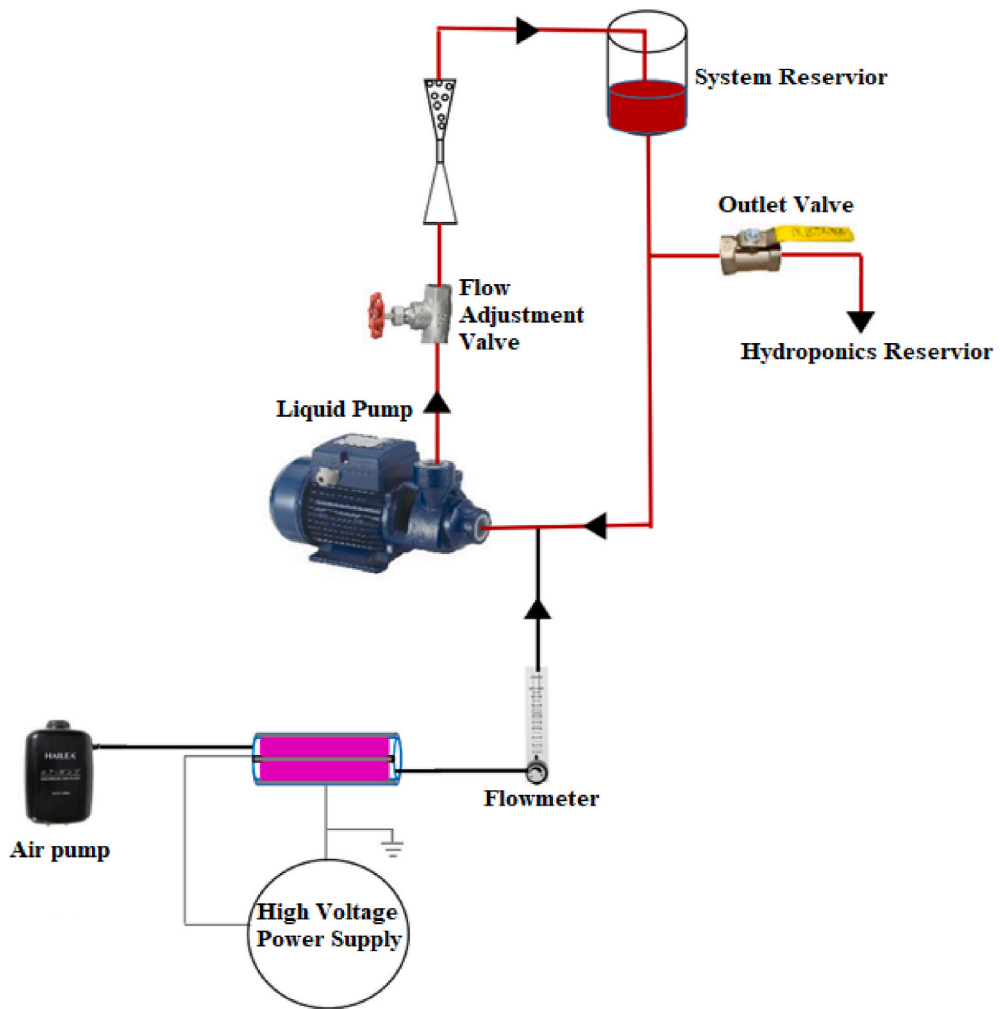


Fig. 2. Schematic of the plasma application system to the gas injected into the nanocavitation reactor.



Fig. 3. A view of the hydroponic culture system in the experiment to investigate the effect of nanobubble and plasma technologies.

### 3. Results and discussion

#### 3.1. Effects of hybrid technology on lettuce yield

First, the results of the comparison of three modes of application of plasma and nanobubble hybrid technology to the solution in the nutrient flow technique will be presented. In the graphs presented in Figs. 4–7, columns with common letters do not have statistically significant differences based on Duncan's test.

Comparison of the duration of 3, 9 and 15 min for the simultaneous application of plasma discharge and nanocavitation technologies showed that the application of hybrid technology for 9 min has significantly increased the yield of lettuce. Although increasing the time from 3 to 9 min had a positive effect, increasing from 9 to 15 min caused a decrease in yield. The reason for this drop can be the increase in the temperature of the solution due to the heat of the nanocavitation, which can cause a decrease in dissolved nitrate due to the activity of nitrate-reducing bacteria. The optimal treatment in this study caused a 59 % and 58 % increase in fresh weight and dry matter of the shoot or head as the edible part of lettuce, respectively. Therefore, it is possible to report a nearly 60 % increase in lettuce yield due to the simultaneous use of plasma and nanobubbles. Also, there were no significant changes in the color of the plants. Another notable thing that was observed was the absence of algae in the 9-min treatment reservoir, while some algae were observed in other reservoirs, as is common in hydroponics.

#### 3.2. Comparison of effects hybrid, plasma and nanobubble treatments on lettuce yield

Following are the comparison results of 1) the application of electric plasma discharge to the air injected into the hydroponic solution by air stone without the use of nanobubbles, 2) the application of nanocavitation to the solution without the use of plasma and 3) the simultaneous use of plasma and nanocavitation as a hybrid technology in Figs. 8–11 are provided. The duration of all three cases was 9 min.

The results showed that the application of each of the plasma discharge and nanocavitation technologies separately did not have a significant effect on increasing performance. But the hybrid technology can bring synergy between these two techniques in hydroponic cultivation.

#### 3.3. Effects hybrid, plasma and nanobubble treatments on lettuce color

Table 2 shows the variations of color values for different studied treatments. In general, noticeable changes of color were not observed. The lightness and greenness of lettuce, as the characteristics desired by the consumer, did not change due to the applied treatments.

#### 3.4. Evaluation of lettuce quality

The amounts of flavonoid, potassium and iron obtained for lettuce treated with hybrid technology for 9 min as optimal treatment and lettuce produced with control or conventional method are compared in Table 3.

In the optimal treatment in terms of yield, an increment in flavonoid metabolites, which usually occurs due to stress, was observed. It is possible that the free radicals produced by plasma and nanocavitation caused the plant reaction to these elicitors and secondary metabolism. Flavonoid as a radical scavenging compound can be a product of secondary metabolism.

An increase in potassium absorption and a decrease in iron absorption have also been observed in the optimal treatment. The decline of iron can be caused by the increase in the pH of the nutrient solution compared to the control. Meanwhile, high amounts of potassium may interfere with the absorption of iron.

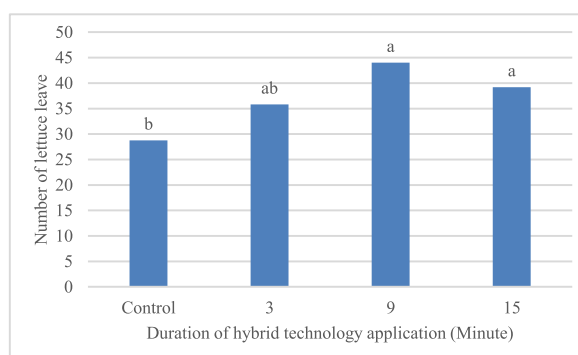
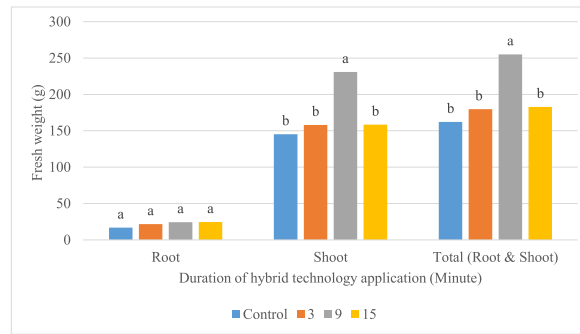
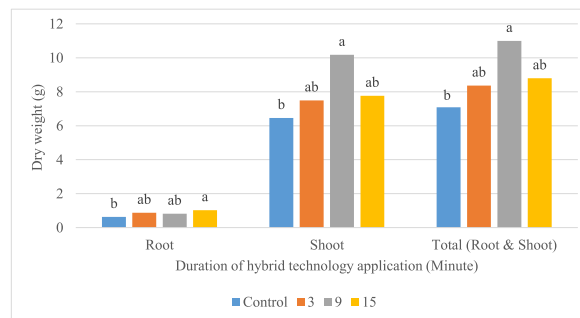


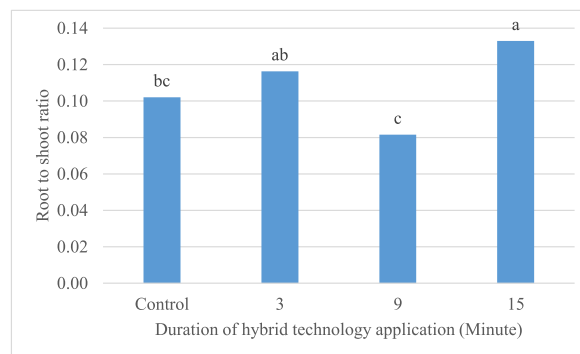
Fig. 4. The effect of time of application of plasma and nanobubble hybrid technology to the nutrient solution of NFT culture on the number of lettuce leaves ( $p < 0.05$ ).



**Fig. 5.** The effect of the time of application of plasma and nanobubble hybrid technology to the nutrient solution of NFT culture on the fresh weight of root, shoot and whole lettuce ( $p < 0.05$ ).



**Fig. 6.** The effect of the time of application of plasma and nanobubble hybrid technology to the nutrient solution of NFT culture on the dry weight of root, shoot and whole lettuce ( $p < 0.05$ ).



**Fig. 7.** The effect of the time of application of plasma and nanobubble hybrid technology to the nutrient solution of NFT culture on the root to shoot ratio ( $p < 0.05$ ).

### 3.5. Discussion

Creation of reactive oxygen and nitrogen species (RONS) is one of the phenomena that occurs when liquid is exposed to plasma. In general, it seems that the effect of PAW on living materials is caused by the synergy of various reactive species. From a practical point of view, in order to obtain the desired effect, identification of the most suitable plasma reactors and optimization of plasma parameters are still needed, followed by scaling up strategies for medium/large scale application [20]. The production of plasma in liquids and for various applications has been the focus of researchers as a new method. But this is difficult because of the very high breakdown voltage. The presence of the gas phase in the liquid, even in small amounts, can be effective in the production and propagation of the pulse electric discharge and the formation of the plasma channel [21]. The purpose of this research was to study the feasibility of improving plasma efficiency in liquid, specifically in hydroponics, due to nanobubbles. In this study, an increase in plasma effectiveness was reported in the presence of nanobubbles. The combination of DBD plasma with systems that produce nanobubbles includes advantages such as increasing the number of radicals or species produced [16]. Also, OH as a chemically active radical and potential antimicrobial

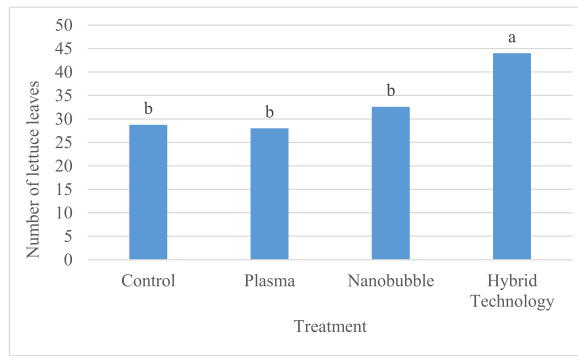


Fig. 8. The effect of plasma and nanobubbles and their combination on the number of lettuce leaves ( $p < 0.05$ ).

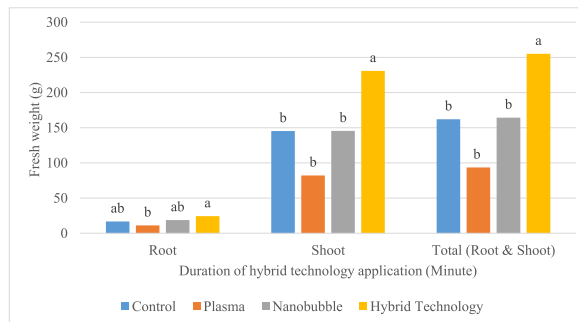


Fig. 9. The effect of plasma and nanobubbles and their combination on the fresh weight of root, shoot and whole lettuce ( $p < 0.05$ ).

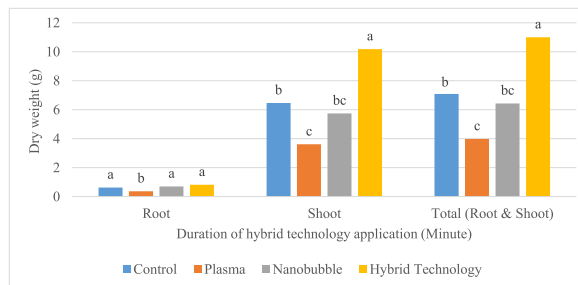


Fig. 10. The effect of plasma and nanobubbles and their combination on the dry weight of root, shoot and whole lettuce ( $p < 0.05$ ).

agent is a product of both plasma and nanobubble technology [8]. Plasma is created by applying energy and mainly by electric field to gas and its ionization, and due to the high contribution of nitrogen and oxygen in the composition of air, if air plasma is applied to water, the formation of reactive species of oxygen and nitrogen, including nitrate, can be expected in water [8]. The role of RNS in agriculture is related to their participation in different signaling pathways in plants that regulate metabolic processes, plant development, response to stress, etc. and therefore can ultimately lead to more germination or an increase in plant growth [20]. Nitrogen is one of the main elements required by the plant, which is usually available through nitrate, and in hydroponic cultivation, it is usually provided through chemical fertilizers. In this research, the hypothesis of treating food solution used in hydroponics with plasma was investigated with the aim of enriching it and replacing chemical substances, but due to the need for stability, absorption and solubility of the products resulting from plasma treatment, nanobubble technology was used. It is worth mentioning that the effect of nanobubbles in improving plant growth was also reported in some studies [3&4]. Electric discharge in both cases with bubbles and without bubbles has led to an increase in nitrate concentration and electrical conductivity, but their higher values were observed in samples containing micro/nanobubbles. This method can be used to form more  $H_2O_2$  and  $NO_3^-$  [14]. Also, the presence of very small bubbles in the flow ensured a larger cross-sectional area, which showed greater contact area and longer residence time compared to macro/micro bubbles. The use of plasma chemical reactions of nanobubbles and the simultaneous presence of liquid and gas in the fluid produced a mixture of active species and ultimately improved the effectiveness of the treatment [16]. The results of the present research showed that the combination of these two technologies can create a promising approach to increase the productivity of hydroponic cultivation



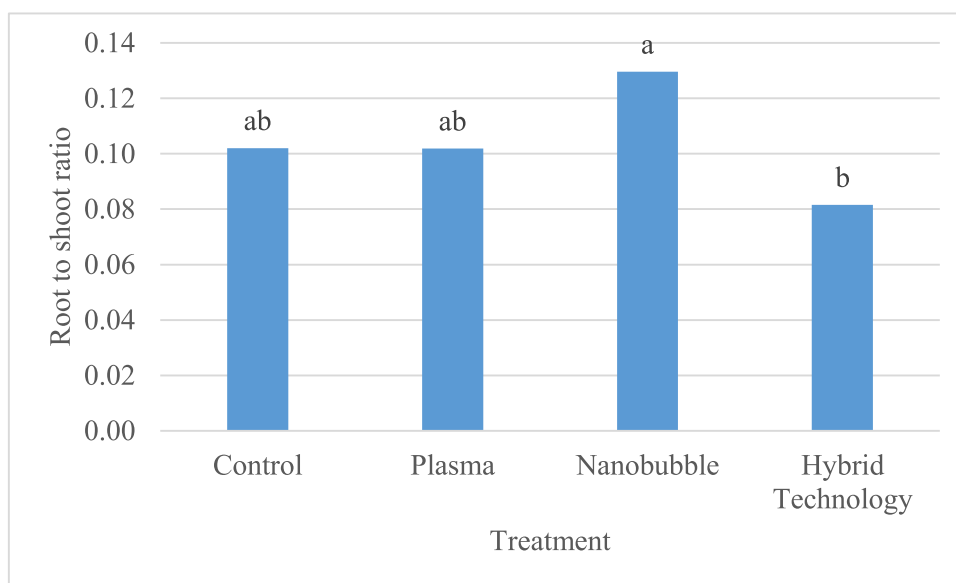


Fig. 11. The effect of plasma and nanobubbles and their combination on the root to shoot ratio of lettuce ( $p < 0.05$ ).

Table 2

The effect of plasma and nanobubbles and different time of their combination on the values  $L^*$ ,  $a^*$  and  $b^*$  of lettuce leaves.

	$L^*$	$a^*$	$b^*$
Control	35.56 a	-17.59 a	18.82 ab
Hybrid technology (3 min)	34.05 a	-15.54 a	16.03 b
Hybrid technology (9 min)	43.25 a	-17.49 a	17.51 ab
Hybrid technology (15 min)	36.93 a	-12.95 a	20.47 a
Plasma	38.14 a	-16.7 a	15.14 b
Nanobubble	34.05 a	-16.89 a	19.34 a

Table 3

Comparison of three nutritional characteristics of lettuce obtained from optimal treatment and control.

	(mg/100 g)Flavonoid	(meq/100 cc)Potassium	(ppm)Iron
Control	30.68	116.8	683.84
Hybrid technology (9 min)	68.7	208.4	467.14

by taking advantage of the benefits of each technology, such as increasing nitrates, antimicrobial compounds, dissolved oxygen, etc.

Algae usually grow strongly in hydroponic systems and cause the pipes and pores to close [18]. In this research, the lack of algae growth in the hybrid treatment tank was also observed, which seems that this new technology will be interesting for treating surface water without using algaecide. The inhibition of algae can be caused by the disintegration of gas bags inside the cell due to hydrodynamic cavitation, stopping the photosynthetic and metabolic activity of the cell due to plasma, hydrogen peroxide produced due to both technologies, and nitrogen and oxygen radicals due to plasma treatment [17]. The properties of plasma-nanobubble hybrid technology make it suitable to prevent or inhibit the pollution of water. The scientific reports in this regard were introduced. Meanwhile some studies conducted grey water footprint assessment for different industries wastewater. They applied treatment scenarios and reuse process [22,23]. It is suggested to implemented similar studies for applying plasma-nanobubble technology to grey water and reusing for nonedible plants cultivation.

Plasma-nanobubble hybrid technology is a cutting-edge innovation combining the benefits of plasma with nanobubble technology in order to improve crop production. The development of specialized equipment capable of producing plasma and nanobubbles at the same time, allowing for better control and modification of the process will be necessary to introduce this hybrid technology into agricultural production. It is possible to use this technology in a variety of applications, such as seed treatment, water treatment, soil sterilization and nutrient supply. Overall, the introduction of plasma-nanobubble hybrid technology in agriculture has great potential to revolutionize production and management by increasing yields as well as a greater sustainability for agricultural practice.

The study may have limitations in terms of generalizability due to variations in plants, nutrient solutions, plasma generation methods and some variables such as input gas flow. Before commercialization and in order to increase reliability, it is necessary to conduct a complementary study with large sample sizes and more replications.

#### 4. Conclusion

In this research, the feasibility study of a hybrid technology including plasma electric discharge and hydrodynamic nanocavitation was carried out and the synergy of these two technologies in hydroponic culture was investigated. Nanobubbles increased plasma efficiency and improvement of hydroponic culture yield (NFT method) was observed. Dielectric barrier discharge (DBD) plasma was a technique used in combination with nanocavitation. Although the combination of these two technologies has recently been noticed in water and wastewater treatment and scientific reports have been presented in this regard, the simultaneous application of these two technologies in hydroponics is considered new. Considering the problem of water crisis and food security in the world, the hybrid technology used can be a significant approach in solving the mentioned problems. In this regard, optimization, troubleshooting and increasing the scale of systems to semi-industrial levels can be considered. Also, the application of this technology in the pasteurization of liquids can also be investigated.

#### CRedit authorship contribution statement

**Rouzbah Abbaszadeh:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Conceptualization. **S. Mohammad Shetab Boushehri:** Data curation.

#### Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Rouzbah Abbaszadeh reports financial support was provided by Payamavaran NanoFanavary Fardanegar Company. Rouzbah Abbaszadeh reports was provided by Payamavaran NanoFanavary Fardanegar Company. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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